Studying some biochemical parameters of *Sarcocystis* parasites isolated from local sheep and goats in Duhok area

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Abstract

Out of 175 heads of sheep, 25 heads were infected with macrocytic *Sarcocystis gigentia* 7%, and out of 25 heads of goats, only 2 had infection 8% with macrocytic *Sarcocystis caprifelis*. Parasitic cysts measurement are 0.8-1.5 cm in sheep and 0.15-0.5 cm in goats, while cystizoits reach in both 0.48-14.8 µm for long types and 1.2- 12.0 µm for short types. Biochemical analysis of 83 *Sarcocystis* parasite cysts (70 cysts were collected from sheep and 13 were collected from goats) were investigated, the total protein (0.849 g/l and 0.813 g/l), Glucose (22.850 mg/dl and 29.268 mg/dl), Cholesterol (50 mg/l and 15.385 mg/l), Triglyceride (18.181 mg/dl and 90 mg/dl), Urea (13.333 mg/dl and 20 mg/dl), Calcium (6.11 mg/dl and 8.80 mg/dl), Sodium (15 mmol./l and 1.5 mmol/l), Potassium (3.875 mmol/l and 3.375 mmol/l), and Iron (31.428 µg/dl and 22.5 µg/dl) for parasite cysts of sheep and goats respectively. Results are differs significantly (P<0.05) for glucose, Cholesterol, triglyceride, urea sodium and iron and that could be attributed to physiological and nature of both kinds of parasite and hosts. Biochemical analysis was done for the first time for *Sarcocystis* parasite of sheep and goats in Duhok area.

دراسة بعض المتغيرات الحيوية لطفيليات الحويصلات الصنوبرية في الضان والمعز المحلي لمنطقة دهوك

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أظهرت 175 رأسا من الضان ان 25 منها مخمجة بالنوع العياني لطفيلي الحويصلات الصنوبرية أظهرت 175 رأسا من الصنوبرية (macrocytic Sarcocystis gigentia) وبنسبة مئوية مقدار ها 7% وكذلك من مجموع 25 رأسا من المعز لوحظ ان 2 فقط مخمجة بالنوع العياني للطفيلي ذاته (macrocytic Sarcocystis caprifelis) وبنسبة 8%. وكانت معدلات قياسات الأكياس الطفيلية هي 0.8 – 1.5 سم بالضان و 0.15 – 0.5 سم بالمعز فيما بلغت أطوال حوينات الكيس 40.48 مايكروميتر للنوع الطولي و 1.2 – 12.0 مايكروميتر للنوع القصيير. أظهرت حوينات الكيس 14.8 – 14.8 مايكروميتر للنوع الطولي و 12.5 مايكروميتر للنوع القصيير. أظهرت التحليلات الكيموحيوية إلى 83 كيس (70 كيس من الضان و 13 كيسا من المعز) تباينا في معدلاتها، إذ كانت 29.268 mg/dl و 18.181 mg/dl و العنسيرول (الشحمين) كانت 1 / 0.813 g و 18.181 mg/dl و الكليسيرات الثلاثية كانت 18.181 mg/dl و 18.80 mg/dl و الكليسيرات الثلاثية كانت 18.375 mmol/l و 18.375 mmol/l و الكليسير عانت 18.375 mmol/l و 18.375 mmol/l والصوديوم كانت 18.428 و 19 للمعنوية إحصائيا (18.40 للعنب والكليستيرول و الكليسيريدات الثلاثية و اليوريا و الصوديوم و الحديد معنوية إحصائيا (18.40 إلى فسلجة وطبيعة كلا من الطفيلي و المضيف.

Introduction

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Sarcocystosis is one of the most worldwide distributed protozoan disease which was infected all mammalian animal and man (1). The parasite life cycle involved two hosts (final or definitive host and an intermediate host) with host specificity in its nature (2). The disease recorded in Duhok area previously with many studies (3, 4, 5). Prevalence of the disease reach high rate in sheep and goats according to local studies (6, 7, 8, 9) or international studies (10, 11, 12). Parasite species reach 150 kinds but the most important one occurrence was seen in small ruminants and that make it economically as well as veterinary public health problems (13, 14). The economic losses related to carcasses exclusion and pathological related manifestation of both final and intermediate hosts (15). Sarcocystis parasite in there intermediate hosts may be macroscopic or microscopic according to parasite species and infection age (12). Sheep and goats infected with macroscopic types belong to species Sarcocystis gigentia, and Sarcocystis caprifelis respectively (15). No previous study at least on Duhok area or in an international publishes deals with biochemical nature of the parasite itself and the current study aims are related with investigate of some biochemical parameters that related to parasite which may interfere with parasite biochemistry and reflect some aspects of physiological and pathological considerations.

Material and Methods

- Collection of samples: Macrocysts of *Sarcocystis* parasite of both sheep and goats were collected from governmental local abattoir of Duhok city after direct examination of slaughtered animals (grossly).
- The most heavily infected organs: (esophagus) of investigated animals had been taken, and transfer with cool box 4°C to the lab were they are photographed.
- Parasite cysts were separated as soon as possible from infected organ photographed, metrical measures were done, cystizoites (zoites cysts contain) observed and measured with aid of ocular micrometer.
- Parasite cysts washed several time with demineralized distilled water, dissection with sterile scissors into small pieces, homogenized with glass type homogenizer (sterile and washed with demineralized water and the squash of parasitic cysts was diluted with sterile distilled demineralized water (1:1) when it was required.
- Squashes of each type of parasitic cyst are prepared separately and they are divided into aliquots (5ml of each), stored by freezing condition (-18 °C).
- Biochemical tests includes total protein, Glucose, cholesterol, triglycerides, Urea Calcium, Sodium, Potassium and Iron were used according to standard method with suitable kits.
- Statistical analysis done with t-paired test (16). Three replicates used for each biochemical test.

Results and Discussion

Biochemical results (Table 1) of both kinds of macroscopic cysts of the parasite isolated from sheep and goats were showed to be as following, Total protein (0.849 g/l. and 0.813 g/l), Glucose (22.850 mg/dl and 29.268 mg/dl), Cholesterol (50 mg/l and 15.385 mg/l), Triglyceride (18.181 mg/d and 90 mg/dl), Urea (13.333 mg/dl and 20 mg/dl), Calcium (6.11 mg/dl and 8.80 mg/dl), Sodium (15 mmol./l and 1.5 mmol/l), Potassium (3.875 mmol/l and 3.375 mmol/l), and Iron (31.428 μ g/dl and 22.5 μ g/dl) for both sheep and goats respectively. Results are differs significantly (P<0.05) for glucose. Cholesterol, triglyceride, urea sodium and iron for that of sheep from that of goats' parasitic cysts. The variety in the biochemical parameters may reflected a kind of enzymatic isomers which involved in that biochemical pathways which could be attributed to genetic developmental variation (17- 21). These biochemical substances

within Sarcocystis parasite could be important for metabolism, physiology, immunology and genetics studies as they can be related to various parasitic isolates through all over the world as an individual variation. Also results indicated for the first time that Sarcocystis parasite have active and different metabolic pathways which may be related to its host in some aspects and that need to be investigate. The study also revealed some biological variation in the occurrence and morphological aspects which may be affected above results. Theses variation showed that macroscopic Sarcocystosis cysts (macrocysts) were varied in there occurrence for sheep or goats as it has been shown with current study. Out of 175 heads of slaughtered sheep (150 heads) and goats (25 heads), there are 83 Sarcocystis parasite cysts (70 cysts were collected from sheep and 13 were collected from goats). The infection rate reach's 7% (11/150) in sheep for Sarcocystis gigente and 8% (2/25) in goats for Sarcocystis caprifeli (Table 2) without any significant difference in that, these results agrees with previous studies (5,6,8). The results of parasite incidence agree with previous studies (5, 8, 9) in observation of macrocytis cysts but differ in the rate of occurrence and that could be attributed to the number of tested samples, availability of final host and related epidemiological factors which varied from area to others. The cysts of parasite were varied in sheep and goats (Fig. 1) in there measures (0.8-1.5 cm in sheep, and 0.15-0.5 cm in goats) with observed significant (P<0.05). Also, cystizoites (Fig. 2) of both kinds of parasite seen to have same measures (0.48- 14.8 µm for long cystizoites and 1.2- 12.0 µm for short one) without significant difference. The variation in parasitic cysts measurements could be discussed as that there is a parasitic individual variation in related to intermediate host (6, 7, 8, 9) and may be related to original dose of infection which related to how these animal taken there feed from ground (15). Also, the cystizoites measurements results may be fixed within various parasitic species as they related to the nature of intrinsic cyst growth within host muscular cells which gave these parasitic cystizoites similarity in all species member due to physiological and natural factors in related to the kinds of parasites and hosts and that may be leads to above biochemical parameters differences as stated above (7).

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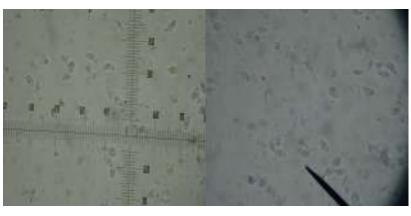
Table (1) Biochemical parameters results for macrocyts of *Sarcocystis spp.* in sheep and goats

| | | 0 | | |
|---------------|-----------------------------------|----------------------|------------|------------------|
| Biochemical | Average- in sheep | Average in goats | Number of | Notes |
| Parameters | $(X \pm S.E.)$ | $(X \pm S.E.)$ | replicates | (significance) |
| Total protein | $0.849 \text{ g/lt.} \pm 0.021$ | 0.813 g/lt. ±0.16 | 3 | not |
| Glucose | 22.850 mg/dlt. ±1.21 | 29.268 mg/dlt±4.09 | 3 | P<0.05 for goats |
| Cholesterol | 50 mg/ lt. ±2.98 | 15.385 mg/lt±0.99 | 3 | P<0.05 for sheep |
| Triglyceride | 18.181 mg/dlt. ± 1.09 | 90 mg/dlt. ±8.04 | 3 | P<0.05 for goats |
| Urea | 13.333 mg/dlt. ± 1.33 | 20 mg/dlt. ±2.88 | 3 | P<0.05 for goats |
| Calcium | $6.11 \text{ mg/dlt.} \pm 0.78$ | 8.80 mg/dlt. ±0.88 | 3 | not |
| Sodium | 15 mmol./lt. ± 1.09 | 1.5 mmol/lt. ±0.33 | 3 | P<0.05 for sheep |
| Potassium | $3.875 \text{ mmol/lt.} \pm 0.55$ | 3.375 mmol/lt. ±0.99 | 3 | not |
| Iron | $31.428 \mu g/dlt. \pm 3.22$ | ±22.5 µg/dlt.v 2.65 | 3 | P<0.05 for sheep |

Table (2) Infection rate of Macrocysts Sarcocystis spp. in native sheep and goats

| Host | Number of examined animals | Number of Macrocysts infection | Percentage of infection rate | Sarcocystis spp. |
|-------|----------------------------|--------------------------------------|------------------------------|------------------------|
| Sheep | 175 | 11 | 7% | Sarcocystis gigentea |
| Goats | 25 | 2 | 8% | Sarcocystis caprifelis |





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Fig. (1) Macrocysts of Sarcocystis spp. (Upper, Fig. (2) Cystizoites of Macrocysts (fresh sheep Sarcocystis gigentea, and Lower, goats preparation) of both sheep and goats (Long and Sarcocystis caprifelis)

short occurrence types) X40

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